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Analysis of Ethanol-Soluble Extractives in Southern Pine Wood by Low-Field Proton NMR

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Abstract: Low-field proton NMR was evaluated as a nondestructive and rapid technique for measuring ethanol-soluble extractives in southern pine wood. Matchstick-sized wood specimens were steeped in extractive-containing solutions to generate extractive-enriched samples for analysis. Decay curves obtained by the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence were analyzed with Contin, a constrained regularization program used to generate continuous distributions of transverse (spin-spin) relaxation times. Air-dry wood samples gave maximum signal amplitudes that did not clearly reflect differences in extractives content. Oven drying removed signals for bound water that were superposed with those for the extractives. Relaxation times and the corresponding amplitudes were then positively correlated with the added extractives. Treatment of air-dry samples with dichloromethane-d_2 prior to analysis increased the mobility of the extractives, thereby resulting in a shift of the corresponding signals to longer relaxation times. This provided the opportunity to quantify the added extractives in the presence of bound water.

Keywords: Extractives, low-field proton NMR, relaxation time, southern pine, wood

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INTRODUCTION

Although wood extractives are nonstructural constituents,[1] they nevertheless can have a significant influence over wood processing parameters as well as the performance of wood-based products. For the pulp and paper industry, high contents of lipophilic extractives in softwood furnishes can raise concerns from increased processing costs to the occurrence of resinous deposits in the paper, especially in the case of high-yield pulps.[2] Lipophilic extractives have also been shown to influence surface wettability,[3] but definitive correlations of lipophilic extractives content to adhesive performance could not be demonstrated.[4] In contrast, the presence of phenolic extractives (e.g., hydrolyzable tannins) has been shown to interfere with the cure of phenolic resin systems.[5,6]

Variability in the extractives content of pine wood, as well as different tree components, has been observed[4,7] and appears to be highly heritable.[8,9] Generally, total extractives contents are determined by extraction with a Soxhlet apparatus, often sequentially with solvents of increasing polarity. Although straightforward and reliable, this methodology can be time consuming and yields a modified (i.e., extractive-free) sample. Rapid determinations of extractives contents have been made through predictions obtained by NIR spectroscopy coupled with multivariate analyses.[10,11] This technique is typically applied to solid wood samples such as increment cores. The resultant values for extractives content on the sample surface are assumed to be representative of the bulk sample. The effect, if any, of extractives migration to the surface, such as that which occurs after cutting,[4] is not known. As an alternative, it is proposed in the current work that a determination of extractives content by low-field proton NMR would be advantageous because it would be nondestructive, rapid, and suitable for the analysis of bulk samples.

Low-field proton NMR has found a niche for assessing the various forms of water (i.e., bound and free water) in wood.[12–14] Applications in other fields of study have shown the utility of this instrumentation for assessing the various forms of water in crop plants,[15] meat,[16] and cheese.[17] In a recent study on Maritime pine wood, non-water transverse (spin-spin) relaxation times were assigned to oleoresin components.[18] The superposition of the extractives and bound water signals in air-dry Maritime pine wood samples was subsequently demonstrated by a novel technique whereby the samples were saturated with dichloromethane-d_2 prior to analysis.[19] This treatment appeared to increase the mobility of the extractives thereby resulting in a shift of the signals derived from the extractives, but not the bound water, to longer relaxation times. Based on these results, the current study was initiated to utilize low-field proton NMR in an examination of southern pine wood samples with known extractive and moisture contents. The former variable was used to determine if this technique could be used to discriminate between, and quantify, the different amounts of extractives present. High and low moisture contents were used to determine if the signals assigned to bound water and

extractives do indeed overlap. It was hypothesized that at very low moisture contents, the signals associated with the bound water would be greatly reduced, revealing the signals associated with the extractives. Furthermore, the proposed increase in relaxation times of the extractives upon treatment with dichloromethane-d_2 was evaluated to determine if the signals associated with bound water and extractives could be separated.

MATERIALS AND METHODS

Preparation of Extractives

Southern pine plywood veneers (3.175 mm thick) were cut into strips (*ca.* 35 mm) across the grain and then split along the grain to obtain matchstick-sized pieces. Some of these were subsequently ground in a Wiley mill equipped with a 10-mesh screen. The resultant wood meal (500 g) was extracted with 95% ethanol (3 L) by steeping at room temperature for 3 days. The extract was dried *in vacuo* to afford an amber resin (17.8 g) that was set aside for later use. FTIR spectra of this resin were collected using a Nicolet Nexus 670 spectrometer equipped with a Thermo Nicolet Smart Golden Gate MKII Single Reflection ATR accessory. For the analysis, a small amount of the resinous material was placed directly on the diamond crystal.

Treatment of Wood Samples with Extractives

Treatment solutions were prepared by dissolving the resinous material in 95% ethanol to achieve concentrations of 5, 10, 15, 20, and 25% (w/w). Solvent alone was used as a treatment representing 0% added extractives. Duplicate samples (2.5–3 g each) of matchstick-sized pieces of wood were completely submerged in aliquots of the treatment solutions (5 g each) for a 3-day period. The glass vials used for treating were tightly capped except when a vacuum was briefly applied with a water aspirator to facilitate the penetration of the treatment solution in the wood. Extractive-enriched wood samples were gently blotted with filter paper, weighed, loosely covered, and allowed to slowly dry under ambient conditions in a fume hood until a stable weight was achieved. One set of the samples was subsequently dried overnight at 75°C. This resulted in sets of air- and oven-dry samples, both previously treated with solutions containing 0–25% extractives. Samples of the untreated wood were also retained to provide a blank for comparison.

Sample Preparation for Low-Field Proton NMR Measurements

Both air- and oven-dry wood samples, at all extractives enrichment levels, were arranged in individual stacks and carefully inserted into borosilicate glass test

tubes (18 × 150 mm). A plastic rod was used to slide each snugly fitting stack down to the bottom of the test tube. The vertically oriented matchstick-sized pieces of wood provided a plug of sample that was of a sufficient height in the test tube (*ca.* 35 mm) as to place the maximum amount of sample within the instrument receiver coil. The average weight of wood loaded into the tubes was 2.55 ± 0.125 g. Each test tube was sealed with a neoprene stopper shortly after the sample was loaded.

Low-Field Proton NMR Parameters

Sample-containing test tubes were inserted into a Minispec mq20 bench-top low-field proton NMR (Bruker Optics, Inc., Rheinstetten, Germany) with a permanent magnet (0.47 tesla field strength, 20 MHz proton) maintained at 40°C. Decay curves obtained by the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence were used for the determination of transverse (spin-spin) relaxation time (T_2) values. For each sample, the pulse separation was 0.05 ms, 128 echoes were collected, and 32 scans were acquired with a 5-s recycle delay. Contin,[20] a constrained regularization program that uses an inverse Laplace transform, was used to resolve the exponentials in the relaxation decay curves to determine transverse relaxation times and the corresponding amplitudes. For quantification purposes, the receiver gain was set using the sample with the highest extractives content for each sample set (i.e., air- or oven-dry). All other samples were analyzed using the same gain so that the amplitudes would be comparable.

Sample Pretreatment with Dichloromethane-d_2

The superposition of the extractives and bound water signals from the low-field proton NMR analysis of the air-dry wood samples was also demonstrated by a novel technique developed by Labbé et al.;[19] superposed signals for the extractives and bound water reflect similarities in the molecular mobility of these two phases. In an attempt to separate these signals, dichloromethane-d_2 (Aldrich) was carefully applied directly to both air- and oven-dry samples (*ca.* 1.3 g per sample) *in situ* using a transfer pipet. This provided solvent-saturated samples without accumulation of excess solvent that could have resulted in the loss of extractives. Samples were analyzed as before with the receiver gain being set using the sample with the highest extractives content.

RESULTS AND DISCUSSION

Extractives Isolation and Characterization

FTIR spectroscopy of the resinous material extracted from the pine wood meal gave spectra predominated by aliphatic C-H (2926, 2856, and 1456 cm^{-1}) and

carbonyl (1717 cm^{-1}) signals typical for pine oleoresin. This expected result simply verified that the extractives were not significantly degraded during isolation or after use for treating the wood specimens. It might be argued the exact site of extractives deposition may differ from that in the native wood; however, this methodology had a significant advantage because it allowed one variable, the extractives content, to be changed. The increase in extractives contents, on a dry-weight basis, ranged from 0.4 to 14% (Table 1). These values compared well with the lipophilic extractives content range of 1 to 14% used for a study on adhesion properties with pine (*Pinus silvestris*) heartwood.[4] Wood samples with different inherent extractives contents covering this range could have been used in our study, but this would have introduced age-related differences (e.g., juvenile versus mature wood) or variability due to differences in growth rate.

Low-Field Proton NMR of Air-Dry Wood Samples

The continuous distributions of relaxation times for a set of air-dry samples are shown in Figure 1. Two peaks were observed in the plot for each sample. The predominant peak in each case had a relaxation time at the maximum amplitude that was between 0.35 and 0.45 ms. These relaxation times were designated as T_{2a}. The relaxation times for the very small peaks ranged from 4 to 38 ms and were designated as T_{2b}. Plots for another set of samples prepared in the same manner gave the same result. Using the results from 3 data sets, the average standard deviation, calculated from the standard deviations for the T_{2a} values at each level of extractives addition, was 3.0% of the mean. Plotting the values for T_{2a} against the increase in extractives content (Figure 2) showed a significant correlation ($R^2 = 0.87$)

Table 1. Extractives and moisture contents for extractive-enriched and control wood samples

Concentration of extractives solution (%)	Extractives content (%)	Moisture content (%)
0	0.4[a]	9.5[c]
5	3.3	9.2
10	6.3	8.7
15	9.1	8.7
20	11.4	8.3
25	14.0	8.3
Blank	NA[b]	8.0

[a]Determined by sample weight change and correction for moisture content.

[b]NA = Not applicable.

[c]Determined on a dry weight basis.

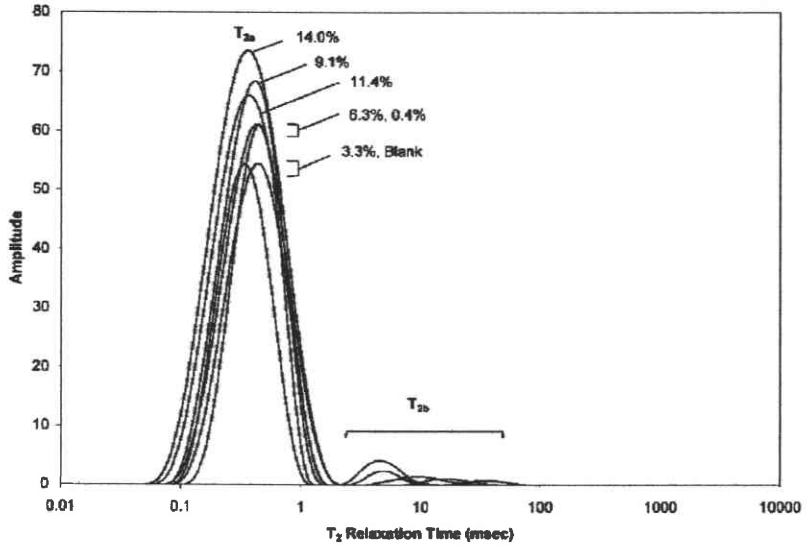

Figure 1. Continuous distribution relaxation time plots for extractive-enriched and control wood samples after air drying (peaks identified by values for extractives content).

with T_{2a} decreasing with the increase in extractives content. Conversely, the T_{2a} amplitudes increased with the increase in extractives content. Given the apparent positive correlation (R^2=0.68) between the T_{2a} amplitudes and the extractives content, the predominant T_{2a} peaks in the continuous distributions of relaxation times (Figure 1) were tentatively assigned, at least in part, to the added extractives component. Coinciding with the weaker correlation for the T_{2a} amplitudes was a higher level of variability with the average standard deviation being 10.3% of the mean. For T_{2b} and the corresponding amplitudes, the plots and the correlations were very similar (T_{2b}, $R^2 = 0.87$; amplitude, $R^2 = 0.66$). The T_{2b} peaks in the continuous distributions of relaxation times were therefore also tentatively attributed to the added extractives component. However, the high degree of variability for these values (T_{2b}, average SD = 41.5% of mean; amplitude, average SD = 25.0% of mean) suggested that any calibrations based on them would be of limited utility.

At this juncture, it should be recognized that the air-dry samples had moisture contents ranging from 8.0 to 9.5% (Table 1) that generally decreased with increasing extractives content. The predominant T_{2a} peaks (Figure 1) were therefore likely from contributions from both the applied extractives and bound water. In a study on paper samples, water in the amorphous phase of cellulose microfibrils was assigned to T_2 values in the range of 0.1 to 0.5 ms.[21]. Because the bound water contributions to the T_{2a} amplitude

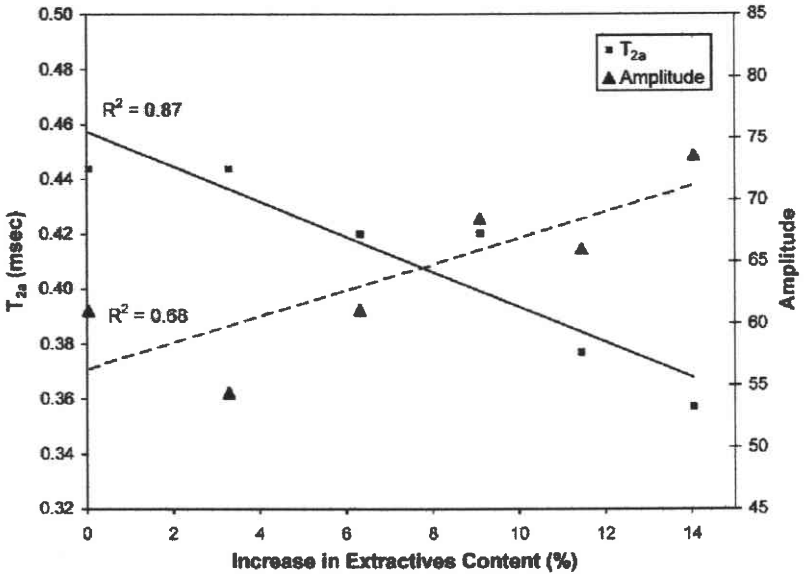

Figure 2. Regression of values for T_{2a} and amplitude with extractives content enrichment: analysis of air-dry wood samples.

would be expected to increase with decreasing extractives content, the data for the air-dry samples were confounded, thus resulting in the relatively weak correlation between the values for T_{2a} amplitude and extractives content. For the aforementioned paper samples, a slow relaxing component at T_2 values of about 9 ms was attributed to residual free water.[21] However, given the low moisture content for our samples, it is unlikely that the T_{2b} peaks we observed would be from free water.

Low-Field Proton NMR of Oven-Dry Wood Samples

To account for the likely signal contributions from bound water, a set of extractive-enriched wood samples was oven dried and analyzed in the same manner as the air-dry samples, albeit using a receiver gain set with the oven-dry sample with the highest extractives content. The resultant continuous distributions of relaxation times (Figure 3) showed a definite trend of peak size increasing along with the increase in the extractives content; plotting the values for T_{2a} ($R^2 = 0.90$) and the corresponding amplitudes ($R^2 = 0.94$) gave strong correlations with the extractives content (Figure 4). This result suggested that signals for bound water and the extractives were indeed superposed in the air-dry samples. We attribute the positive correlation between T_{2a} and the extractives content to the ability to detect what is likely greater extractives mobility coinciding with a greater amount of extractives within the cell wall

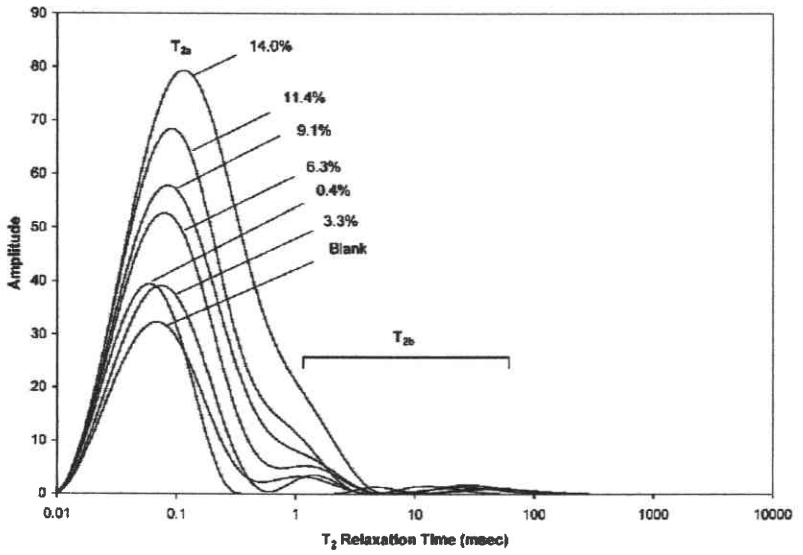

Figure 3. Continuous distribution relaxation time plots for extractive-enriched and control wood samples dried at 75°C (peaks identified by values for extractives content).

matrix. Given the relative size of the T_{2a} peak for the sample without added extractives, it also became apparent that along with the extractives and bound water, the T_{2a} peak appears to include fast relaxing components from the solid matrix. Contributions from protons in the solid phase should be constant because the weight of wood was essentially the same for each analysis and all samples originated from the same sheet of veneer. It is possible that the mobility of the protons in the solid phase may decrease upon the removal of bound water. Thus, the magnitude of the signal overlap between the solid phase and the extractives may change depending upon the moisture content. Nevertheless, because contributions from the solid phase did not have a detrimental effect on the correlations with the extractives content, such as that imparted by bound water, any superposition of the signals from the solid matrix and extractives components appears to be of little consequence for an extractives content determination by this technique.

Plotting the values for T_{2b} and the corresponding amplitudes for the very small peaks in continuous distributions of relaxation times showed essentially no correlations (T_{2b}, $R^2 = 0.38$, amplitude, $R^2 = 0.06$) with the extractives content. It therefore seems unlikely that the components responsible for these signals are directly related to the extractives component. Although it is possible that these signals are artifacts from the analysis, we cannot eliminate the possibility that they are simply derived from yet unidentified components. However, at this stage it is obvious that they do not appear to interfere with our ability to use the predominant T_{2a} signals for extractives content determinations.

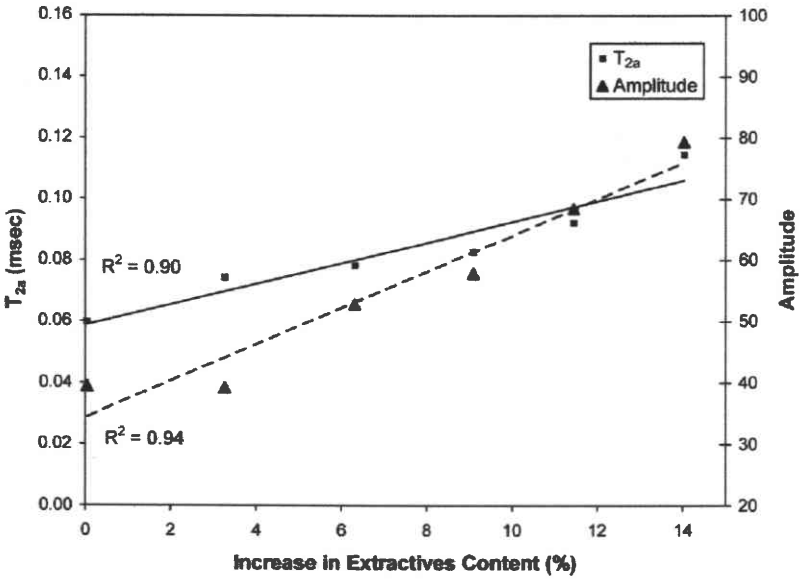

Figure 4. Regression of values for T_{2a} and amplitude with extractives content enrichment: analysis of wood samples dried at 75°C.

Low-Field Proton NMR of Wood Samples Saturated with Dichloromethane-d_2

The superposition of the extractives and bound water was also demonstrated by a novel technique developed by Labbé et al.[19] whereby samples were saturated with dichloromethane-d_2 prior to analysis. This treatment appears to increase the mobility of the extractives thereby increasing their relaxation times. The immiscibility of water and the organic solvent likely facilitates this signal separation. The continuous distributions of relaxation times (Figure 5) showed a significant increase in the T_{2b} amplitudes relative to the still predominant T_{2a} peaks (<0.5 ms). Plots for both T_{2a}, and the corresponding amplitudes, showed no significant correlation with extractives content (T_{2a}, $R^2 = 0.04$; amplitude, $R^2 = 0.40$). Repeated analyses ($n = 4$) of the sample with the highest extractives content showed that the values for T_{2a} and the corresponding amplitudes were stable (T_{2a}, average SD = 2.7% of mean; amplitude, average SD = 6.8% of mean). We attribute these signals to a relatively constant moisture content (Table 1) and contributions from the solid matrix. Whereas the values for T_{2b} also showed a poor correlation ($R^2 = 0.50$) with the extractives content (Figure 6), the corresponding amplitudes were strongly correlated ($R^2 = 0.95$). This suggested that sample saturation with dichloromethane-d_2, and determinations of the T_{2b} amplitudes, may permit the quantification of lipophilic extractives in pine

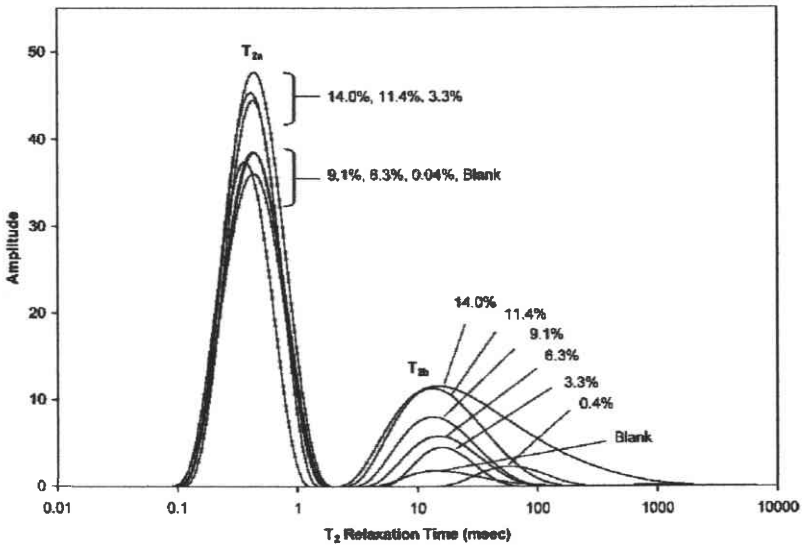

Figure 5. Continuous distribution relaxation time plots for extractive-enriched and control wood samples after air drying and saturation with dichloromethane-d_2 (peaks identified by values for extractives content).

wood while in an air-dry condition. For T_{2b}, and the corresponding amplitudes, the standard deviations were 25.8% and 14.3% of the respective means. Methodology refinements need to be explored to determine the extent that the variability in the amplitude values can be reduced.

Analogous results were obtained for a set of oven-dry samples treated with dichloromethane-d_2. As before, continuous distributions of relaxation times (Figure 7) showed the sizes of the T_{2b} peaks to increase along with the increase in the extractives content (T_{2b}, $R^2 = 0.70$; amplitude, $R^2 = 0.98$). In contrast to the air-dry samples, the sizes of the T_{2a} peaks for the oven-dry samples were inversely proportional to the extractives treatment levels (T_{2a}, $R^2 = 0.79$; amplitude, $R^2 = 0.55$). In the absence of water, and the presence of only a very small extractives component, all that remained to be detected were mostly solid matrix components. The sample treated with ethanol alone, and that not treated with ethanol (i.e., the blank control), both show a large T_{2a} peak and a small T_{2b} peak. For those samples with a higher extractives component, the smaller T_{2a} peaks and larger T_{2b} peaks demonstrate a greater signal contribution from the extractives than the solid matrix.

Together, the continuous distributions of relaxation times for the air-dry (Figure 5) and the oven-dry (Figure 7) samples show that bound water contributes to the T_{2a} peaks in the air-dry samples and that the extractives are represented by the T_{2b} peaks in both the air-dry and oven-dry samples. This

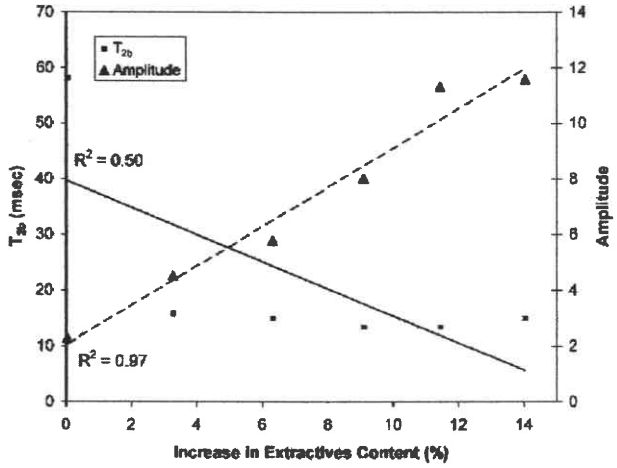

Figure 6. Regression of values for T_{2b} and amplitude with extractives content enrichment: analysis of air-dry wood samples saturated with dichloromethane-d_2.

verified that there was a separation of the superposed signals from the extractives and bound water components in the air-dried samples. Repeated analyses ($n = 5$) of an extractive-enriched oven-dry sample gave values for T_{2a} and the corresponding amplitudes which were much more variable (T_{2a}, average

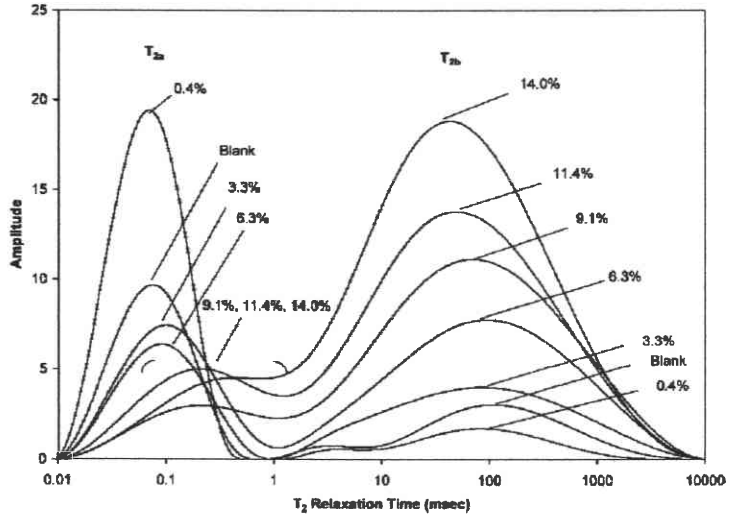

Figure 7. Continuous distribution relaxation time plots for extractive-enriched and control wood samples after oven drying and saturation with dichloromethane-d_2 (peaks identified by values for extractives content).

SD = 59.1% of mean; amplitude, average SD = 30.1% of mean) than observed with the air-dry samples. We attribute this to the low signal level resulting from the presence of only trace amounts of bound water and the low, if any, contribution from the solid matrix. For T_{2b} and the corresponding amplitudes, the standard deviations were 21.7% and 3.2% of the respective means. Given this result, it would appear that the use of the T_{2b} amplitudes for extractives determinations would show greater reproducibility with decreasing moisture content.

These results further demonstrate that the treatment with dichloromethane-d_2 expands the versatility of the low-field proton NMR technique for characterizing and quantifying lipophilic extractives contents by increasing their mobility to afford a slower relaxing component, represented by T_{2b}, now separated from fast relaxing components (i.e., bound water, solid matrix).

CONCLUSIONS

Analysis of pine wood samples enriched with ethanol-soluble extractives by low-field proton NMR demonstrated the potential of this technique for the quantification of lipophilic extractives in wood. Oven drying of the wood samples prior to analysis removes signals for bound water that are superposed with those for the extractives. In the continuous distribution relaxation time plots, this results in the relaxation times at the maximum amplitudes, as well as the amplitudes, to change with the extractives contents. Treatment of samples with dichloromethane-d_2 further supported the hypothesis that extractives and bound water signals were superposed in the continuous distribution relaxation time plots. Saturation with dichloromethane-d_2 results in a separation of signals for bound water and lipophilic extractives. Thus, deuterated solvents of low polarity appear to provide a means to separate low-field proton NMR signals thereby expanding the options for characterizing and quantifying wood constituents in the presence of bound water.

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